1813

Some Organic Sulfur Compounds in Vegetables and Fodder Plants and their Significance in Human Nutrition

BY PROF. DR. ARTTURI I. VIRTANEN [*]

LABORATORY OF THE FOUNDATION FOR CHEMICAL RESEARCH, BIOCHEMICAL INSTITUTE, HELSINKI, (FINLAND)

Many plants that serve for human nutritional purposes contain compounds from which physiologically active substances are formed by enzymic reactions. Usually such compounds, and the enzymes which react with them, are located in different cells, so that the active substances are formed only on crushing the plant. Examples are the lachrymatory factor and the antimicrobial substances formed from the different cysteine-S-oxides of the onion and garlic, as well as the goitrogenic compounds formed from the thioglucosides occurring in the Brassica species.

Introduction

Belief in the preventive or curative effect of vegetables towards disease has persisted for thousands of years. Garlic, onion, cress, and cabbage are examples. In Eastern Europe and Asia Minor, large amounts of raw onion or garlic are a normal feature of the diet. The science of nutrition has paid but little serious attention to these beliefs, since, to some extent, they can be explained on the basis of the vitamins and minerals contained in these vegetables, and because other alleged effects, if they exist, seem to be connected with substances better regarded as drugs than foods.

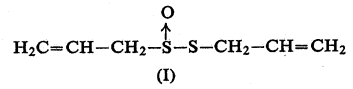
Many vegetables contain precursors from which physiologically active substances (such as flavor compounds, antibiotics or anti-thyroid substances) are formed enzymically when the plants are crushed. In the intact plant, precursor and enzyme are separated from each other in different cells [1]; the enzymic reaction is not possible before the cells are crushed. If the enzymes in intact plants are destroyed by heating, or by placing the plants for some time in cold alcohol, the active substances are no longer formed upon crushing and it is possible to isolate the precursor from the plant. Fresh and cooked vegetables thus differ essentially. This is the situation, for example, with regard to the onion and its relations, and in plants of the *Cruciferae* family.

Sulphur Compounds from the Onion and Garlic

Allicin and Alliin

At the end of last century, the ill-smelling oils obtained by steam distillation from various *Allium* species were investigated and found to contain alkyl sulfides.

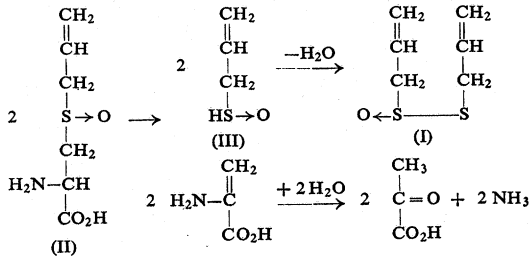
Eighteen years ago, *Cavallito* et al. [2] succeeded in elucidating the chemical nature of an antibiotic factor formed in crushed garlic as a result of enzyme action. They proposed the structure (I) and called the product allicin:



(I)

The substance has only a mild, not too unpleasant garlic odour. It is relatively unstable. The allyl disulfide and trisulfide present in garlic oil, are formed from it by secondary reactions.

Some years later, *Stoll* and *Seebeck* [3] succeeded in isolating the precursor of allicin from garlic. This proved to be (+)-*S*-allylcysteine-*S*-oxide or alliin (II); it is decomposed by the alliinase present in garlic as follows:



Scheme 1. Formation of allicin from alliin by allinase

We found no peak of mass 90 corresponding to allylsulfenic acid in mass-spectral studies of the degradation products of crushed garlic. It is therefore possible that allylsulfenic acid (III) disproportionates immediately to allylsulfinic acid (IV) and allyl mercaptan (V). Allicin (I) might then be formed from these compounds by elimination of water (Scheme 2). *Challenger* [4] has also considered this kind of reaction possible.

According to *Stoll* and *Seebeck*, prerequisites for the activity of alliinase are that the cysteine derivative

[*] Lecture held on October 12th. 1961 at the Fifth Organic Chemistry Conference on Organic Sulfur Compounds at Natick, Massachusetts. (Sponsored by the National Academy of Sciences – National Research Council).

The investigations reported in the lecture are part of a research project under U. S. Public Law No. 480, 83rd. Congress. The investigations on the composition of onion were first supported by a grant from the Rockefeller Foundation.

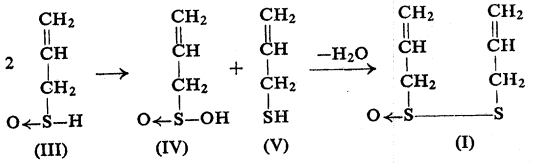
[1] *L. Guignard*, C. R. hebd. Séances Acad. Sci. *111*, 249 (1890).

[2] *C. J. Cavallito, J. S. Buck*, and *C. M. Suter*, J. Amer. chem. Soc. *66*, 1952 (1944).

[3] *A. Stoll* and *E. Seebeck*, Helv. chim. Acta *32*, 197 (1949); Advances in Enzymol. *11*, 377 (1951).

[4] *F. Challenger:* Aspects of the Organic Chemistry of Sulfur. Butterworths, London 1959, p. 56.

should be in the form of the *S*-oxide, and that the group linked to the sulfur atom should be aliphatic. *S*-Benzylcysteine-*S*-oxide is not degraded. In experiments in our laboratory, *S*-benzylcysteine-*S*-oxide has been degraded with a purified enzyme preparation from onion. The



Scheme 2. Formation of allicin (I) from allylsulfenic acid (III)

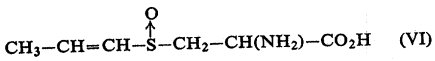
enzymes from garlic and onion differ somewhat with regard to the influence of pH on their activity and stability, but both show the highest degree of reactivity with the allyl derivative as substrate, and both split only *S*-oxides. Pyridoxal phosphate stimulates both enzymes. An enzyme which splits *S*-alkylcysteines as well as *S*-oxides has been isolated by *Gmelin* et. al. [5] from the seeds of *Albizzia lophanta*.

Synthetic *S*-allyl-L-cysteine-*S*-oxide is racemic at the sulfur atom and *Stoll* and *Seebeck* have separated the diastereoisomers by fractional crystallization. The dextrorotatory component proved to be identical with natural alliin and was quantitatively and rapidly degraded by the enzyme, whereas the laevo isomer was decomposed much more slowly. Derivatives of D-cysteine were not attacked.

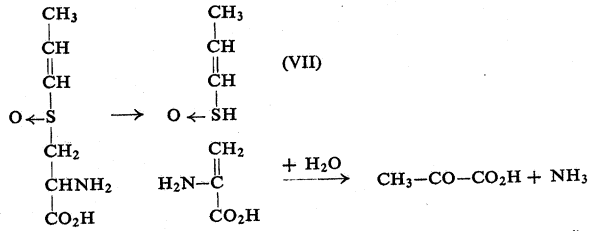
Matikkala and *Virtanen* [6] found *S*-methyl and *S*-n-propyl-L-cysteine-*S*-oxides to be precursors of alkylthio alkylsulfinates corresponding to (I). These sulfinates also exert an antibiotic effect on many gram-positive and – negative bacteria, yeasts, and fungi [7]. Their effect is, however, not so great as that of allicin. No allylcysteine-*S*-oxide could be found in onion.

The Lachrymatory Substance of the Onion

Recently, *Spåre* and *Virtanen* [8] found that the crystalline precursor of the lachrymatory factor in the onion is also an *S*-alkenyl-cysteine-*S*-oxide. On paper chromatograms the compound behaved like alliin, and had the same elementary composition, but no lachrymatory factor was formed enzymically from alliin. Present data indicate that the precursor of the lachrymatory factor in the onion is (+)-*S*-(prop-1-enyl)-L-cysteine-*S*-oxide (VI).



Pyruvic acid and ammonia were observed in equivalent amounts as end-products of the enzymic cleavage of (VI). In mass-spectral studies of an aqueous solution of (VI) and a dialyzed enzyme preparation from onion, a strong peak of mass 90 was observed, but no peak of higher mass [9]. A weaker peak of mass 73 was also found. This could have arisen through cleavage of a hydroxyl group from the compound of mass 90. In this connection, it ought to be mentioned that a peak of mass 61 is formed from dimethyl sulfoxide (mass 78). The lachrymatory factor may thus be prop-1-enylsulfenic acid (VII) (mass 90), formed from *S*-(prop-1-enyl)-L-cysteine-*S*-oxide (Scheme 3).

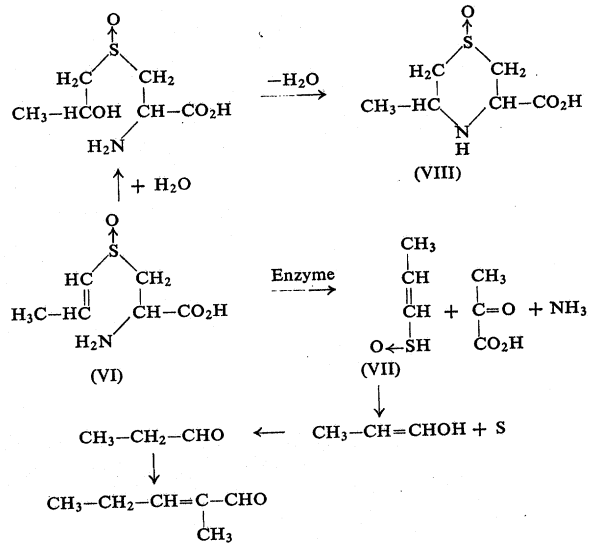


Scheme 3. Formation of *S*-propenylsulfenic acid from *S*-(prop-1-enyl)-L-cysteine-S-oxide

No aliphatic sulfenic acids are known, but the position of the double bond in (VII) is a possible stabilizing factor. The lifetime of the lachrymatory factor is short and hence its chemical investigation is difficult.

In their earlier mass-spectral analysis of flavor compounds in onion *Niegisch* and *Stahl* [10] observed a peak of mass 90 in the −80 °C cold traps and proposed the structure HO–CH_2–CH_2–CHS (β-hydroxypropanthial). The lachrymatory precursor is now characterized chemically as (VI), hence the older structure is untenable.

A large amount of propionaldehyde is formed in the enzymic cleavage of (VI), probably spontaneously from the first decomposition product, prop-1-enylsulfenic acid. 2-Methyl-2-pentenal was also formed in smaller amounts in crushed onion, probably from propionaldehyde [11]. The products of enzymic and chemical cleavage of *S*-(prop-1-enyl)-cysteine-*S*-oxide are presented in Scheme 4.



Scheme 4. Products of enzymatic and chemical cleavage of *S*-propenylcysteine-*S*-oxide (VI)

[5] *R. Gmelin, G. Hasenmaier*, and *G. Strauss*, Z. Naturforsch. *12b* 687 (1957).

[6] *A. I. Virtanen* and *E. J. Matikkala*, Acta chem. scand. *13*, 1898 (1959).

[7] *L. V. Small, J. H. Bailey*, and *C. J. Cavallito*, J. Amer. chem. Soc. *69*, 1710 (1947).

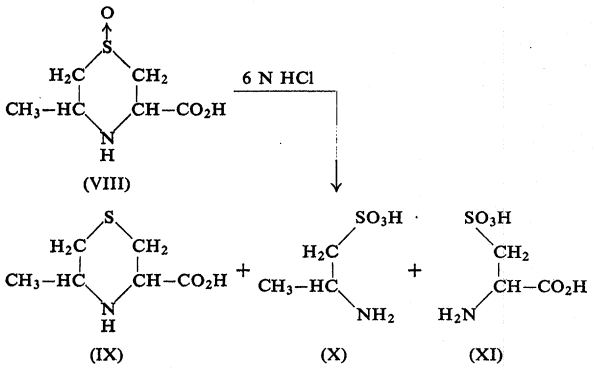
[8] *A. I. Virtanen* and *C.-G. Spåre*, Suomen Kemistilehti B *34*, 72 (1961).

[9] *T. Moisio, C.-G. Spåre*, and *A. I. Virtanen*, Suomen Kemistilehti B *35*, 29 (1962).

[10] *W. D. Niegisch* and *W. H. Stahl*, Food Res. *21*, 657 (1956).

[11] *A. I. Virtanen* and *C.-G. Spåre*, Suomen Kemistilehti B *34*, 18 (1961); *C.-G. Spåre* and *A. I. Virtanen*, Acta chem. scand. *15*, 1280 (1961).

Some years ago a new cyclic sulfoxide, $C_6H_{11}O_3NS$ (VIII), was isolated from onion by *Matikkala* and *Virtanen* [12,13]. When heated with 6 N HCl, it disproportionates to give 2-methyltaurine (X) and cysteic acid (XI) as oxidation products; as well as the thioether (IX) as reduction product (Scheme 5).

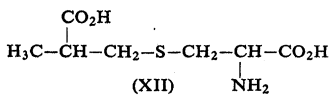


Scheme 5. Disproportionation of cycloalliin (VIII)

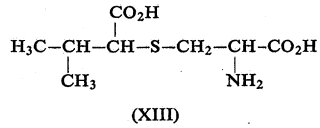
On the basis of these and other findings, it was inferred that the compound is 3-methyl-1,4-thiazane-5-carboxylic acid-1-oxide (VIII) [14,15]. This structure was confirmed by synthesis from *S*-allylcysteine [15]. Because (VIII) has the same elementary composition as alliin, the name cycloalliin was proposed. At that time, the isomer (VI) of alliin – the precursor of the lachrymatory factor – was not yet known, and it was thought that (VIII) might be formed in onion from alliin [15]. Later it was shown that the lachrymatory precursor cyclizes to cycloalliin when the pH of its solution is raised above 7. It is, therefore, probable that the lachrymatory precursor in the onion is the precursor of cycloalliin (see Scheme 4) [8]. Cycloalliin can be formed during the isolation but it is present even in intact onions, as was proved by boiling the vegetable material in 6 N hydrochloric acid whereupon the thioether (IX) is formed [16]. *S*-(Prop-1-enyl)-cysteine-*S*-oxide (VI) decomposes even in 0.5 N hydrochloric acid, and is not cyclized in acid solution.

γ-Glutamyl-Peptides

Another type of cysteine derivative was found in the γ-glutamyl peptide isolated from onion by *Matikkala* and *Virtanen* [17]. Its structure was proved to be (−)-*S*-[β-carboxyprop-1-yl]-L-cysteine (XII).



This was confirmed by synthesis from L-cysteine and β-bromoisobutyric acid. *Mizuhara* and *Oomori* [18] have since found this compound in human urine in both the free and bound forms. According to their private communication the presence of the amino acid in urine cannot be traced back to the consumption of onions. The same authors [19] have isolated another cysteine derivative, *S*-(1-carboxy-2-methylprop-1-yl)-cysteine (XIII), from the urine of hyper-cholesterolemic patients suffering from arteriosclerosis or myxodema.



Some years ago, our attention was attracted to the presence of numerous peptides in onions. These were hydrolyzed even with dilute hydrochloric acid (0.5–1 N) at 100 °C. All proved to be γ-glutamyl peptides. Ten years ago the only known representative of this group was glutathione. The peptides were fractionated on a Dowex 1 column with 1 N acetic acid followed by 1 N hydrochloric acid. Isolation of individual peptides was accomplished on a cellulose powder column with butanol-acetic acid-water. So far, nine γ-glutamyl peptides have been isolated in crystalline form from onion and their structures have been elucidated [20,21]. In the order of elution, from Dowex 1, these peptides are:

1. γ-L-Glutamyl-L-valine
2. γ-L-Glutamyl-L-isoleucine
3. γ-L-Glutamyl-leucine
4. γ-L-Glutamyl-*S*-(prop-1-enyl)-cysteine-*S*-oxide
5. Ethyl ester of γ-glutamyl peptide No. 9 (possibly artifact)
6. γ-L-Glutamyl-L-methionine
7. γ-L-Glutamyl-*S*-methylcysteine
8. γ-L-Glutamyl-L-phenylalanine
9. γ-L-Glutamyl-*S*-(2-carboxyprop-1-yl)-cysteinylglycine.

Some difficulty was experienced in determining the structure of peptide No. 4. Its cysteine residue was decomposed by even the mildest acid hydrolysis. However hydrolysis of the peptide was eventually affected with an enzyme preparation from calf kidney and the cysteine derivative isolated in crystalline form identified as *S*-(prop-1-enyl)-cysteine-*S*-oxide (VI) [21]. It had the same dextrorotation as the sulfoxide (VI), which occurs in free form in onions. Hence it was not formed from the thioether by oxidation during isolation.

[12] *A. I. Virtanen* and *E. J. Matikkala*, Suomen Kemistilehti B *29*, 134 (1956).

[13] *E. J. Matikkala* and *A. I. Virtanen*, Suomen Kemistilehti B*30*, 219 (1957).

[14] *A. I. Virtanen* and *E. J. Matikkala*, Suomen Kemistilehti B*31*, 191 (1958).

[15] *A. I. Virtanen* and *E. J. Matikkala*, Acta chem. scand. *13*, 623 (1959).

[16] *A. I. Virtanen* and *E. J. Matikkala*, Suomen Kemistilehti B *34*, 114 (1961).

[17] *A. I. Virtanen* and *E. J. Matikkala*, Hoppe-Seylers Z. physiol. Chem. *322*, 8 (1960).

[18] *S. Mizuhara* and *S. Oomori*, Arch. Biochem. Biophysics *92*, 53 (1961).

[19] *S. Oomori* and *S. Mizuhara*, Biochem. biophysical Res. Commun. *3*, 343 (1960), and personal communication.

[20] *A. I. Virtanen* and *E. J. Matikkala*, Suomen Kemistilehti B*34*, 53 (1961).

[21] *A. I. Virtanen* and *E. J. Matikkala*, Suomen Kemistilehti B*34*, 84 (1961).

Numerous γ-glutamyl peptides are also present in garlic, many being the same as those from onion. However, some other peptides are also present. We have isolated one of these as a crystalline compound and shown it to be γ-glutamyl-*S*-allyl-L-cysteine [22]. It was later isolated independently by *Suzuki* [23].

The function of the γ-glutamyl peptides in onion and garlic is not well understood. Since the peptides are present in both bulbs and seeds, but not, or only scantily, in the green parts of the plants, they are probably reserve substances. When the green leaves push out from the bulbs, the γ-glutamyl peptides disappear rapidly and thus probably take part in nitrogen metabolism. There is, therefore, even a physiological similarity between glutamine and γ-glutamyl peptides. It is peculiar that in onions we have found neither γ-glutamyl transferase nor an enzyme able to hydrolyze the γ-peptide bond. Enzymes of both types are present in kidney and liver [24].

Meister [25] demonstrated the existence of a transaminase specific for glutamine. We obtained small amounts of alannei from pyruvate and γ-glutamyl-*S*-(prop-1-enyl)-cysteine by the action of a dialyzed enzyme preparation from onion leaves at pH 7.4 [26]. An enzyme preparation made from rat liver had a stronger effect than the onion preparation [25]. It is still uncertain, however, whether this is the principal pathway for the metabolism of γ-glutamyl peptides in onions.

Biological Action of Cysteine-S-Oxides

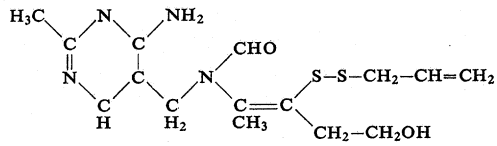
At least three of the cysteine-*S*-oxide derivatives, *S*-methyl-, *S*-propyl-, and *S*-allyl-, may act as antibiotics in animals and humans because of the antimicrobial properties of the alkyl and alkenyl thiosulfinates formed from them. These compounds inhibit the growth of *Staphylococcus* and many other bacteria in dilutions of 1:10000 to 1:100000. The allyl compound has the highest activity.

The antibiotic effects of onions vary greatly depending especially on the variety, but also on climatic conditions. Homogenates of onions grown in Finland can be diluted with 10 to 15 volumes of water and still inhibit the growth of *Staphylococcus* for 24 hours; garlic homogenates have an activity ten to twenty times higher. However, the antibiotic potency of these vegetables in the human organism cannot be calculated solely on the basis of the results of experiments *in vitro* because of the incomplete decomposition of the alkyl sulfoxides by chewing and the ease with which the alkyl thiosulfinates are reduced to disulfides. Although these also have an antibiotic effect against *Staphylococcus*, it is weaker than that of the corresponding thiosulfinates. A large part of the *S*-alkyl sulfoxides probably pass unchanged into the intestinal canal. It is possible that they are decomposed there, forming thiosulfinates and disulfides. In our experiments with *Coli*-type intestinal bacteria, cleavage of *S*-allyl- and *S*-propylcysteine-*S*-oxide was demonstrated [27] by the presence of the corresponding disulfides. It is therefore probable that regular consumption of garlic and onion may have an antibiotic and regulative effect on the bacterial flora of the intestinal canal. This might then account for their curative or preventive effect on intestinal disturbances.

No antibiotic effect is exhibited by aqueous solutions of the lachrymatory factor (VI) from onions. If raw onions are eaten, this factor will also be formed in the mouth and some even in the stomach and intestinal canal. Irritating and stimulating effects on the mucous membrane are then possible.

Nearly thirty years ago, *v. Kokas* and *v. Ludány* [28] found in experiments with dogs and pigeons that the rhythm of the intestinal villi was greatly increased by crushed garlic and onion in concentrations of 1:1000. At the same time glucose resorption was enhanced by 15 to 20 %. Cloves, paprika, and pepper had a similar effect. These unique experiments were performed *in vivo* as well as with pieces of intestine. Unfortunately they have never been repeated. *Von Euler* has reported that an intratumoural injection of aliin in Jensen and benzpyrene sarcomas in rats even leads, in some cases, to the disappearance of the tumour [28a].

Fujiwara et al. [29] have reported that allithiamine and its propyl homologue are more active than thiamine when given to rats on a vitamin B_1-free diet and more curative when given to B_1-deficient animals. *Yurugi* [29] has found that allithiamine (XIV) is formed in the reaction between thiamine and allicin. Methyl- and propyl thiosulfinates react similarly with thiamine. Because allithiamine and its homologues are more readily absorbed from the intestine than thiamine, alkyl- and alkenyl thiosulfinates may be of some importance in the "activation" of thiamine in the intestines.



(XIV)

The amounts of sulfur compounds isolated to date in this laboratory from the onion are given in Table 1. Taken together, they amount to some 80–90 % of the soluble sulfur content.

Table 1. Approximate concentrations of the sulfur compounds so far isolated from the onion [mg./kg. fresh-weight]

S-Propylcysteine-*S*-oxide	50
S-Methylcysteine-*S*-oxide	200
S-Propenylcysteine-S-oxide	40
Cycloalliin [a]	2500
γ-L-Glutamyl-(+)-*S*-propenyl-cysteine-*S*-oxide	1300
γ-L-Glutamyl-*S*-methylcysteine	50
γ-L-Glutamylmethionine	50
S-(2-Carboxyprop-1-yl)glutathione	330

[a] A part of the cycloalliin may be formed from *S*-propenylcysteine-*S*-oxide during elution of amino acids with ammonia from the Amberlite column.

[22] *A. I. Virtanen* and *I. Mattila*, Suomen Kemistilehti B *34*, 44 (1961).

[23] *T. Suzuki, M. Sugii*, and *T. Kakimoto*, unpublished.

[24] *C. S. Hanes, F. J. Hird*, and *F. A. Isherwood*, Biochem. J. *51*, 25 (1952); *F. J. Hird* and *P. H. Springell*, ibid. *56*, 417 (1954).

[25] *A. Meister* and *S. V. Tice*, J. biol. Chemistry *187*, 173 (1950).

[26] *A. I. Virtanen* and *E. J. Matikkala*, unpublished.

[27] *M. Saarivirta* and *A. I. Virtanen*, unpublished.

[28] *E. v. Kokas* and *G. v. Ludány*, Arch. exp. Pathol. Pharmakol. *169*, 140 (1933).

[28a] Cf. *A. Stoll* and *E. Seebeck*, Advances in Enzymol. *2*, 377 (1951).

[29] Cf. *B. C. Johnson*, Ann. Rev. Biochem. *24*, 419 (1955).

Mustard Oil Glucosides

Mustard oil glucosides are characteristic of many plants, especially the *Cruciferae*, but also *Tropaeolaceae*, *Resedaceae*, and certain other families. Many of the mustard oils (isothiocyanates) formed enzymically from these glucosides when the plants are crushed have a characteristic, pungent, but not unpleasant, flavor. The antimicrobial activity of some mustard oils is high with respect to molds and fungi and fairly high against many bacteria (see Figure 1) [30].

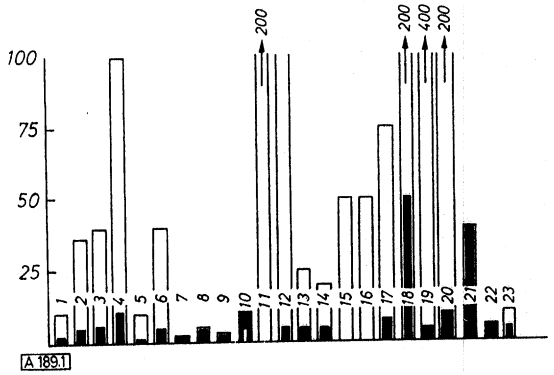


Fig. 1. Lowest inhibition-concentrations (μg./ml.) of different mustard oils, some of their precursors, and other compounds.
White area: Activity against *Staphylococcus aureus* 209 Innsbruck
Black area: Activity against *Penicillium glaucum*.
Ordinate: Minimum concentration required for inhibition [μg/ml].

Isothiocyanates:

1. Methyl-
2. Ethyl-
3. Propyl-
4. Isopropyl-
5. Allyl-
6. Phenyl-
7. Benzyl-
8. Ethylphenyl-
9. *m*-Methoxybenzyl-
10. *p*-Methoxybenzyl-
11. *p*-Hydroxybenzyl- (Sinalbin)
12. Methylthiopropyl-
13. Methylthiobutyl-
14. Methylthiopentyl-
15. Methylsulfinylpropyl- (Glucoiberin)
16. Methylsulfonyl
17. Methylsulfonylbutyl-

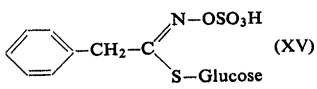
Thiocyanates:

18. Isopropyl-
19. Benzyl-
20. Ethylphenyl-

Other sulfur compounds:

21. Dibenzylcarbothialdin
22. Na-Benzyldithiocarbamate
23. Benzylammonium-benzyldithiocarbamate

Benzyl isothiocyanate has the highest antibiotic effect of all the known mustard oils when the activity against both fungi and bacteria is considered. Its precursor, glucotropaeolin (XV), occursin *Lepidium sativum* and *Tropaeolum majus*, both used as vegetables in Western Europe.



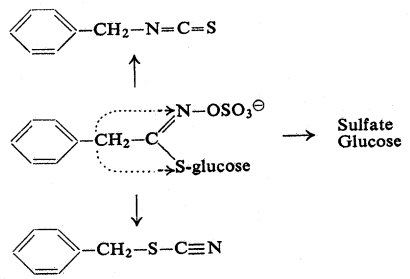
(XV)

Winter [31] studied their antibiotic effect in the human organism and found that even 20 to 30 g causes a high antibiotic activity in the urine for many hours against *Staphylococcus* and *E. coli*. An extract of *Tropaeolum* is used as a remedy against infections.

[30] *M. Saarivirta*, unpublished.

[31] *A. G. Winter*, Hippokrates *28*, 695 (1957).

In the course of further investigations on an isothiocyanate fraction from crushed moist *Lepidium sativum* seeds, *Gmelin* and *Virtanen* [32] separated the fraction into benzyl isothiocyanate and benzyl thiocyanate. Mainly benzyl thiocyanate is found in crushed seeds of *Lepidium ruderale* but only benzyl isothiocyanate in those of *Tropaeolum majus*. In all cases glucotropaeolin (XV) was decomposed during the enzymic cleavage. In the green parts of *Lepidium* species benzyl isothiocyanate and thiocyanate are also formed enzymically. The formation of benzyl thiocyanate suggests that after enzymic liberation of glucose and sulfate, rearrangement of the remainder of the molecule can take place in two ways, *i.e.* the benzyl group can migrate to either the nitrogen or the sulfur atom (Scheme 6).



Scheme 6. Enzymatic degradation of glucotropaeolin (XV)

Benzyl isothiocyanate and benzyl thiocyanate differ greatly in their properties. The latter has a very slight antibiotic effect upon bacteria, but affects the iodide uptake of the thyroid gland, whereas benzyl isothiocyanate has no such effect.

The seeds of *Thlaspi arvense*, *Peltaria* species, and *Brassica nigra*, which contain the allyl thioglucoside sinigrin form only allyl thiocyanate after crushing [32].

It is not yet clear why the enzymic cleavage of the mustard oil glucosides by myrosinase leads to the formation of isothiocyanates in most plants and thiocyanates, or both, in others. *Gaines* and *Goehring* [33] have shown that myrosinase is a mixture of two enzymes, one of which splits off glucose and the other sulfate. The possibility that the relative activities of these enzymes in different plants might not be the same seems to offer, at first sight, a plausible explanation to our findings. However, a preparation of myrosinase (purified only by dialysis) formed benzyl isothiocyanate alone from crystalline glucotropaeolin, whereas the crushed and moistened seeds of *Lepidium ruderale* gave mainly benzyl thiocyanate.

Cabbage plants, and many other *Cruciferae*, contain *S*-methylcysteine-*S*-oxide [34,35] but lack an enzyme like alliinase. Hence, no thiosulfinate is formed in these plants after crushing. Degradation of methylcysteine-*S*-oxide is, however, possible in the intestinal canal.

Glucobrassicin

Over thirty years ago, *Chesney* et al. [36] observed that rabbits fed on a diet of cabbage developed enormous goiters which were largely reversible with iodide. In some

[32] *R. Gmelin* and *A. I. Virtanen*, Acta chem. scand. *13*, 1474 (1959).

[33] *R. D. Gaines* and *K. J. Goehring*, Biochem. biophysical Res. Commun. *2*, 207 (1960).

[34] *C. J. Morris* and *J. F. Thompson*, Chem. and Ind. (London) 951 (1955).

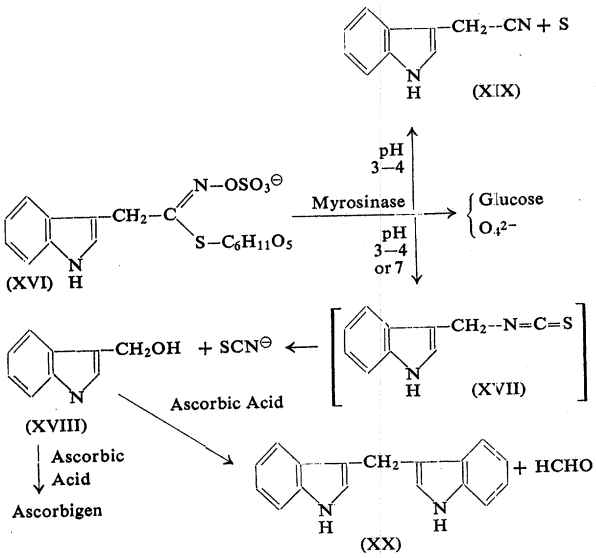
[35] *R. L. M. Synge* and *J. C. Wood*, Biochem. J. *60*, 15 (1955).

[36] *A. M. Chesney*, *T. A. Clawson*, and *B. Webster*, John Hopkins Hosp. Bull. *43*, 261 (1928).

regions, where sections of the community regularly consume large amounts of cabbage, this type of goiter is not uncommon. The goitrogenic factor was unknown for many years.

Barker's [37] discovery that goiters developed in two patients who had undergone prolonged treatment with potassium thiocyanate for hypertension drew the attention of investigators to the thiocyanate ion (SCN^-) as a goitrogenic factor. Until recently it was thought that this ion must be formed endogenously in the human organism, *i.e.* from organic cyanides, nitriles, and cyanogenic glucosides in food. In 1958, *Michajlovskij* and *Langer* [38] found considerable amounts of thiocyanate in cabbage and the authors regarded this anion in food as the cause of the goitrogenic effect. Somewhat later, *Gmelin* and *Virtanen* [39] showed that intact cabbage contains no thiocyanate but a precursor which liberates SCN^- when cabbage is crushed. The precursor was isolated in crystalline form from *Brassica oleracea sabauda* and *gongyloides* as the tetramethylammonium salt [40,41]. The chemical composition as well as the nature of enzymic and chemical decomposition products demonstrate the presence of a thioglucoside which we call glucobrassicin; this proved to be an indole derivative (XVI) [41,42].

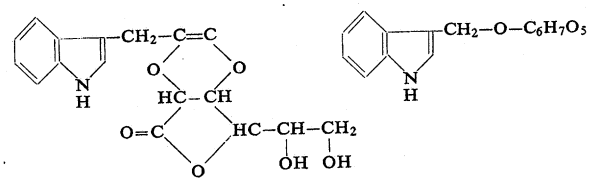
Glucobrassicin is the first mustard oil glucoside with an indole group in the molecule and is the precursor of many biologically interesting compounds (Scheme 7). 3-Indolylmethyl isothiocyanate (XVII), which should be formed from glucobrassicin by the action of myrosinase at a neutral pH, is immediately split into two fragments, SCN^- and 3-hydroxymethylindole (XVII). The formation of indolylacetonitrile (XIX) and sulfur at pH 3.5 is probably due to the occurrence of a chemical reaction after the enzymic liberation of glucose and sulfate. This reaction explains the chemical nature of the



Scheme 7. Products formed by the enzymatic degradation of glucobrassicin (XVI)

"bound growth-hormones" in *Brassica* plants studied by many earlier investigators [43]. 3-Indolylacetic acid is formed in the acid and alkaline hydrolysis of glucobrassicin.

3,3'-Diindolylmethane (XX) is formed from two molecules of 3-hydroxymethylindole by splitting off one molecule of formaldehyde. The 3-hydroxymethylindole can also react with ascorbic acid present in cabbage to form ascorbigen. This compound has been isolated by *Procházka* et al. [44] from cabbage and is believed to be an original substance in this plant. The formula (XXI) previously proposed for ascorbigen has to be corrected. The synthesis of ascorbigen [42] from 3-hydroxymethylindole and ascorbic acid shows that the ascorbic acid in ascorbigen is linked with the hydroxymethyl group of the indole compound, probably by an ether bridge (XXIa). Ascorbic acid is split off during hydrolysis with dilute hydrochloric acid. Ascorbigen administered orally to guinea pigs, or man, has an antiscorbutic effect [45].



Nagashima et al. [46] have reported that ascorbic acid strongly stimulates the activity of myrosinase.

In addition to glucobrassicin, we [42] recently isolated *N*(1)-methoxy-glucobrassicin (neoglucobrassicin) from rutabaga. It is also present in small amounts in some cabbage species. Thiocyanate ion is split off quantitatively from this compound by the action of myrosinase at pH 7. Table 2 illustrates the amounts of thiocyanate formed by myrosinase in some *Brassica* species [39].

Table 2. SCN^- content in cabbage species after myrosinase treatment

Brassica species	SCN^- formation per 100 g. fresh plants
Brassica oleracea [*] var. *sabauda* ssp. *Ulmer*	27–31 mg.
Brassica oleracea [*] var. *gemmifera*	10 mg.
Brassica oleracea [*] var. *capitata*	4 mg.
Brassica oleracea [*] var. *cretica*	4 mg.
Brassica napus [+] var. *rapifera* [+]	8.8 mg.
Brassica napus [*] var. spring rape	2.5 mg.
Brassica rapa [*] var. winter turnip rape	1.7 mg.

[*] leaves [+] root.

[37] *M. H. Barker*, J. Amer. med. Assoc. *106*, 762 (1936).

[38] *N. Michajlovskij* and *P. Langer*, Hoppe-Seylers Z. physiol. Chem. *312*, 26 (1958); *P. Langer* and *N. Michajlovskij*, ibid. *312*, 31 (1958).

[39] *R. Gmelin* and *A. I. Virtanen*, Acta chem. scand. *14*, 507 (1960).

[40] *R. Gmelin*, *M. Saarivirta*, and *A. I. Virtanen*, Suomen Kemistilehti B *33*, 172 (1960).

[41] *R. Gmelin* and *A. I. Virtanen*, Suomen Kemistilehti B *34*, 15 (1961).

[42] *R. Gmelin* and *A. I. Virtanen*, Ann. Acad. Sci. fennicae Ser. A. II.. No. 107 (1961).

[43] *H. Linser*, Planta *29*, 392 (1939); *J. A. Bentley*, Ann. Rev. Plant Physiol. *9*, 47 (1958).

[44] *Ž. Procházka*, *V. Šanda*, and *F. Šorm*, F. Coll. czech. chem. Commun. *22*, 654 (1957).

[45] *M. Kiesvaara* and *A. I. Virtanen*, Acta chem. scand. *16*, 510 (1962).

[46] *Z. Nagashima* and *M. Uchiyama*, Bull. agric. Soc. Japan *23*, 555 (1959).

When large amounts of cabbage are regularly consumed, the iodine content of the diet should be higher. Some SCN^- is split off from glucobrassicin even on boiling in water (Fig. 2) [42].

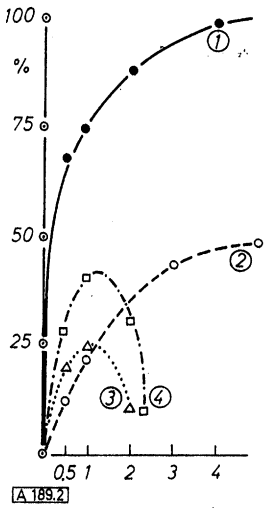
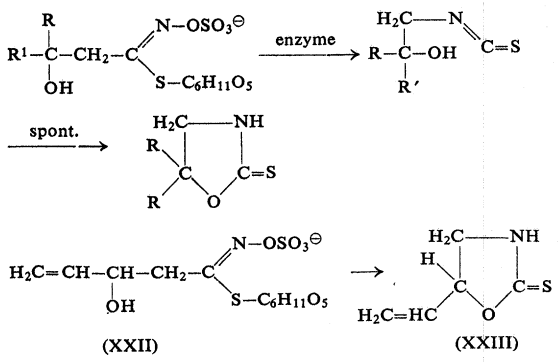


Fig. 2. Formation of thiocyanate from glucobrassicin (1) through enzymatic splitting, (2) through the action of boiling water, (3) by boiling in 0.1 N HCl and (4) by boiling in 0.1 N NaOH. Ordinate: liberated SCN^- [% of theoretically possible amount] Abscissa: Time [hours]

Goitrin and Progoitrin

The thiooxazolidones represent another type of antithyroid substances formed enzymically from precursors in cruciferous plants. They inhibit the synthesis of thyroid hormones and this inhibition is not reversed by iodine. More than ten years ago, *Astwood*, *Greer* and *Ettlinger* [48] isolated goitrin, L-5-vinyl-2-thiooxazolidone, from moistened, crushed *Brassica* seeds as well as from crushed rutabaga. This compound had about the same antithyroid acitivity as the drug propylthiouracil, used therapeutically against hyperthyroidism.

Thiooxazolidones are cyclic derivatives of isothiocyanates containing a hydroxyl group at the C-2 position. After *Ettlinger* and *Lundeen* [49] had shown the correct structure of the mustard oil glucosides it was at once possible to see that goitrin is formed from its precursor, progoitrin (isolated by *Greer* [50]), as shown in Scheme 8.



Scheme 8. Formation of thiooxazolidone from mustard oil glucosid and of goitrin (XXIII) from progoitrin (XXII)

The relatively abundant occurrence of progoitrin in many *Brassica* seeds is the explanation of the finding[51] that seeds of cabbage and rape are goitrogenic to rats. Progoitrin could, at first, not be found in the green parts of *Brassica* plants, *e.g.* cabbage or kale. Some years ago, however, the formation of goitrin in crushed cabbage, kale, green rape, *etc.* [52–54] was demonstrated.

The amount of progoitrin in cabbage is so low – the highest value found in our laboratory was about 20 μg./g. of fresh plants – that goitrin cannot influence the function of the thyroid gland if cabbage is used as a vegetable in reasonable quantities. Higher values have, however, been found [53] in various fodder plants belonging to the genus *Brassica*.

Because of the large amounts of fodder which cows eat daily, cruciferous plants in feedstuffs could conceivably make milk goitrogenic. *Clements* and *Wishart* [55] alleged that in Tasmania, and some other parts of Australia, milk produced on a feed composed largely of marrow kale actually has goitrogenic properties which cannot be prevented by iodine. Because of the far-reaching implications of this statement, we have studied the problem more thoroughly. The transfer of goitrin from fodder to milk was found to be very low. Feeding experiments were carried out using crystalline goitrin, crushed and moistened rape seed, green rape, and marrow kale [47, 56] and, at the most, only 0.05 % of the goitrin fed was to be found in the milk. When crystalline progoitrin was administered without *Brassica* plants, only traces of goitrin were to be found in the milk. The goitrin content of the milk could not be raised to a level anywhere near that needed to make milk goitrogenic.

The transfer of thiocyanate to milk was also studied [57]. It seems impossible to raise the SCN^- content of cow's milk over approximately 10 mg. per liter even with large amounts of *Brassica* plants in the feed. With cows fed on grass, hay, and cereals, the SCN^- content of the milk varies between 2 and 5 mg./liter.

Our experiments with both volunteers and rats [47] led us to the conclusion that milk has no goitrogenic properties even when cows are on widely different diets, including mainly various *Brassica* plants.

Benzoxazinone Glucosides from Grain Seedlings

Brief reference should be made to a new group of substances found in young rye, wheat and maize plants. These compounds do not contain sulfur, but they are

[47] Cf. *A. I. Virtanen*, Experientia *17*, 241 (1961).

[48] *E. B. Astwood, M. A. Greer*, and *M. G. Ettlinger*, J. biol. Chemistry *181*, 121 (1949).

[49] *M. G. Ettlinger* and *A. J. Lundeen*, J. Amer. chem. Soc. *78*, 4172 (1956).

[50] *M. A. Greer*, J. Amer. chem. Soc. *78*, 1260 (1956).

[51] *C. E. Hercus* and *H. D. Purves*, J. of Hyg. *36*, 182 (1936).

[52] *A. I. Virtanen, M. Kreula*, and *M. Kiesvaara*, Acta chem. scand. *12*, 580 (1959).

[53] *M. Kreula* and *M. Kiesvaara*, Actachem. scand. *13*, 1375 (1959).

[54] *M. R. Altamura, L. Long*, and *T. Hasselstrom*, J. biol. Chemistry *234*, 1847 (1959).

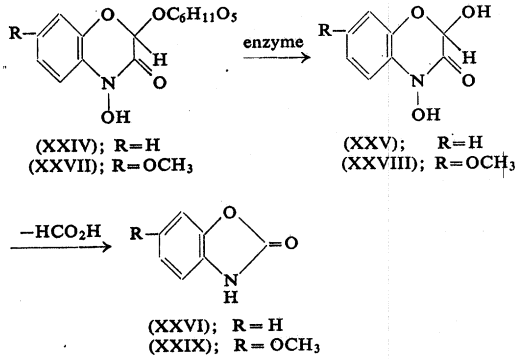
[55] *F. W. Clements* and *J. W. Wishart*, Metabolism *5*, 623 (1956).

[56] *A. I. Virtanen, M. Kreula*, and *M. Kiesvaara*, Acta chem. scand. *13*, 1043 (1959).

[57] *A. I. Virtanen* and *R. Gmelin*, Acta chem. scand. *14*, 941 (1960).

hydroxylamine derivatives (like the mustard oil glucosides). Because they are not present in seeds they do not occur in human, but only in animal nutrition.

Relatively large amounts of glucoside (XXIV) [58–61] are present in young rye plants, and of glucoside (XXVII [62] in wheat and maize plants. The glucosides are rapidly hydrolyzed when the plants are crushed, and the aglucones (XXV) and (XXVIII) are formed. Both have an antifungal and antibacterial effect. Experiments with ^{14}C-labelled compounds have shown that an intramolecular redox reaction occurs, in which formic acid is split off and the six-membered heterocycle is converted into a five-membered ring with a reduced nitrogen



(XXIV); R=H
(XXVII); R=OCH_3

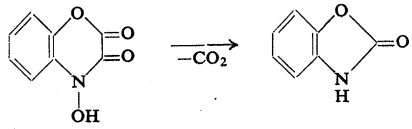
(XXV); R=H
(XXVIII); R=OCH_3

(XXVI); R=H
(XXIX); R=OCH_3

Scheme 9. Benzoxazinone glucosides from rye (XXIV) and wheat or maize plants (XXVII)

atom [63]. This reaction only takes place if the hydroxyl group is linked to the nitrogen atom, and if the bond between the carbon and oxygen atoms in the heterocyclic ring is weak (see Scheme 9).

Accordingly, benzoxazolinone (XXVI) is formed from 2,4-dihydroxy-1,4-benzoxazin-3-one (XXV) and, with liberation of CO_2, from 4-hydroxy-1,4-benzoxazine-2,3-dione (XXX).



(XXX) (XXVI)

The isolation of this new group of compounds shows how easily mistakes may arise in work of this nature. While investigating the rye plant for substances which might explain the different resistance of several rye varieties to *Fusarium nivale*, benzoxazolinone (XXVI) was isolated by *Hietala* and *Virtanen* [64]. The corresponding 6-methoxy derivative (XXIX) was isolated [65] from maize and wheat plants. At first we believed, erroneously, that these compounds existed as such in the plants, since they cannot form *O*-glucosides. This error was later corrected and the precursors were isolated. Many compounds in the literature believed to be present in the intact plants, may in reality be artifacts formed during the crushing of the plants or, in some cases, at a later stage of the isolation process.

[58] *A. I. Virtanen* and *P. K. Hietala*, Suomen Kemistilehti B *32*, 38, 138, 252 (1959).

[59] *A. I. Virtanen* and *P. K. Hietala*, Acta chem. scand. *14*, 499 (1960).

[60] *P. K. Hietala* and *A. I. Virtanen*, Acta chem. scand. *14*, 502 (1960).

[61] *E. Honkanen* and *A. I. Virtanen*, Acta chem. scand. *14*, 504 1214 (1960).

[62] *Ö. Wahlroos* and *A. I. Virtanen*, Acta chem. scand. *13*, 1906 (1959).

[63] *E. Honkanen* and *A. I. Virtanen*, Acta chem. scand. *15*, 221 (1961).

[64] *A. I. Virtanen* and *P. K. Hietala*, Acta chem. scand. *9*, 1543 (1955).

[65] *A. I. Virtanen*, *P. K. Hietala*, and *Ö. Wahlroos*, Suomen Kemistilehti B *29*, 143, 171 (1956).